Albumin-mediated Transport of Rose Bengal by Perfused Rat Liver

KINETICS OF THE REACTION AT THE CELL SURFACE

E. L. FORKER and B. A. LUXON, *Department of Medicine, University of Missouri-Columbia, Missouri 65212*

ABSTRACT Rapid dissociation of organic anions from plasma albumin maximizes the presentation of free ligand to the cell surface and thus favors its efficient hepatic extraction. Even assuming these optimal conditions, however, taurocholate and rose bengal have hepatic extraction fractions that are higher than can be accounted for by spontaneous dissociation of their albumin-ligand complexes. In this study we developed a transport model that attributes this behavior to sites on the hepatocyte plasma membrane that bind the albumin-ligand complexes, promoting the transport of ligand into the hepatocyte. Fitting this model to rose bengal removal rates measured over a wide range of albumin concentrations yields estimates of the number of cell surface sites and their affinity for albumin. These estimates are in good agreement with those reported by Weisiger, Gollan, and Ockner for the binding of ligand-free albumin to isolated hepatocytes. We conclude that both experiments measure the same phenomenon and, accordingly, that the binding of albumin to the cell surface is the functional equivalent of albumin-mediated transport.

INTRODUCTION

The liver removes a diverse group of organic anions from the circulation with remarkable efficiency, despite extensive binding of these solutes to albumin. Proceeding from the assumption that the binding reaction in extracellular fluid is fast enough to be considered at equilibrium, we have argued (1, 2) that the removal of free ligand is much faster than can be accounted for by spontaneous dissociation of the albumin-ligand complex and, accordingly, that there must be an intrahepatic mechanism for removing the albumin-bound form of the ligand. Weisiger et al. (3) have made a similar proposal based on a different approach to analyzing the kinetics of organic anion removal. Because albumin diffuses readily to the cell surface, a plausible, though unverified, postulate is that albumin-ligand complexes interact with the cell surface, presenting additional ligand to the transport site. Because albumin itself is not removed in this process, however, free ligand must be the form that ultimately engages the transport carrier. The chemistry and geometry of these interactions are unknown, but it is clear that if the phenomenon is to confer any physiologic advantage, ligand liberated from albumin on the cell surface must engage the transport system without mixing with the pool of free ligand present in extracellular fluid.

Although direct evidence for this idea has not appeared, two observations suggest that there are only a limited number of sites on the cell surface available to mediate the transport of albumin-bound complexes. First, when the ligand extraction fraction is measured in the presence of increasing concentrations of albumin, the removal process displays competitive inhibition, even though the concentration of bound ligand is unchanged (2). Second, ligand-free albumin binds reversibly to isolated rat hepatocytes. The equilibrium constant for this reaction (25 μM albumin) has been reported by Weisiger et al. (3), as well as the number of sites per cell (10^7).

The rationale for designing the present experiments is as follows. If binding of albumin-ligand complexes to the cell surface mediates ligand removal, it should be possible to predict the number of cell surface sites and their affinity for albumin from measurements of ligand extraction carried out over a wide range of albumin concentrations. If the model that leads to these predictions is realistic, these estimates should be similar

Received for publication 14 February 1983 and in revised form 11 July 1983.

to those reported by Weisiger. Thus, our estimates, which depend on the capacity of the putative binding mechanism to enhance ligand removal by functionally intact liver, are to be compared with those obtained by Weisiger from the association of ligand-free albumin with isolated hepatocytes. The new data show that both the number of sites and their affinity for albumin are similar to those reported by Weisiger—a result that supports the kinetic model we have assumed and suggests that the association of ligand-free albumin with isolated liver cells is the same process that mediates the removal of albumin-bound ligand by the liver lobule.

METHODS

Hepatic perfusion. 26 rat livers (Sprague-Dawley females, liver weight $\simeq$ 8 g) were perfused in situ with oxygenated Krebs-Ringer bicarbonate buffer containing glucose (270 mg/dl), trace amounts of ^{131}I-labeled rose bengal (Mallinckrodt Corp., St. Louis, MO), and various concentrations of bovine albumin (97% globulin free, Sigma Chemical Co., St. Louis, MO). The concentration of rose bengal in afferent perfusate was constant in any particular experiment, but in each rat we measured its hepatic extraction at each of two albumin concentrations. Alternate livers were perfused with the lower albumin concentration first. The paired observations yielded 52 sets of data for analysis. 7 of the 26 rats are the subject of an earlier report (2), in which the perfusion procedure is described in detail. In these earlier experiments the concentration of rose bengal was varied between 5×10^{-9} and 5×10^{-8} M to verify that in this range the hepatic extraction fraction of rose bengal is independent of its concentration. In the remaining 19 experiments the concentration of rose bengal was 10^{-8} M.

The perfusions were carried out in the nonrecirculating mode, at a fixed flow of 32 ml min^{-1}. This flow rate (approximately four times normal) was selected to ensure adequate oxygen delivery in the absence of erythrocytes. The extraction fraction of rose bengal was determined from the mean concentration of radioactivity in eight samples of hepatic venous effluent collected over 16 min, following an initial interval of 4 min required to establish the steady state. Perfusate containing dissolved albumin was clarified by filtration, but the albumin itself was not further purified. Protein concentrations were measured spectrophotometrically from the absorbance at 280 nm or by the method of Bradford (4). Labeled rose bengal contained <2% free iodine as supplied by the manufacturer. It was not refined further or diluted with carrier. An automated crystal detector was used to measure the gamma emission of ^{131}I.

Protein binding. The free fraction of rose bengal in perfusate was determined spectrophotometrically from the spectral shift associated with the binding of this dye to albumin. The details of this procedure and the binding kinetics have been reported previously (2). Briefly, we determined the absorbance peak and the corresponding extinction coefficient for the bound and free forms of the dye. The free fraction at any particular albumin concentration was then determined from the absorbance at each of these wavelengths (550 and 565 nm).

Kinetic modeling. The kinetic model is based on assumptions outlined here. The governing equations are derived in the Appendix.

We assume that only free ligand can engage the carrier for hepatic uptake and that the efflux and excretion steps are independent of ligand binding to perfusate albumin. The model superimposes on this conventional formulation a uniform distribution of sites on the cell surface that reversibly bind perfusate albumin whether or not the albumin carries one or more ligand molecules. Each cell surface site has the property that if it binds an albumin-ligand complex, the ligand it contains is subject to transport. Uptake occurring by this pathway is governed by the same transport rate constant that governs removal of free ligand directly from extracellular fluid. The total rate of ligand removal is thus proportional to the mass of ligand that is free in extracellular fluid plus the mass that is bound to the cell surface as albumin-ligand complexes. This formulation, while undoubtedly oversimplified, is mathematically tractable and it avoids ad hoc assumptions that would otherwise be required to model the way in which surface-bound complexes present their ligand to the transport carrier. The reaction of ligand with albumin is assumed to be fast compared with the uptake process and compared with convection and diffusion in extracellular fluid. Rose bengal binding is thus assumed to be effectively at equilibrium. The binding of albumin to the cell surface is at equilibrium by definition because in the steady state there is no net removal of protein from extracellular fluid and because the cell surface sites are assumed to have the same affinity for albumin-ligand complexes as they do for the free protein.

Assuming that the kinetic performance of the whole liver can be satisfactorily approximated by a single equivalent sinusoid, the solution to the governing differential equation is

$$-F \ln (1 - E) = v\bar{K}[\alpha + (1 - \alpha)\lambda]/[1 - (1 - \alpha)\lambda]$$

$$\lambda = S_t/(S_t + K_s + P), \quad (1)$$

in which E is the steady-state extraction fraction of rose bengal, α is its free fraction, F is perfusate flow, and P is the albumin concentration in extracellular fluid. The unknown parameters, S_t and K_s, are, respectively, the moles of cell surface sites per unit of extracellular volume and the equilibrium constant for the reaction between albumin and these sites. The remaining parameter, $v\bar{K}$, incorporates the first order rate constants for uptake, efflux, and excretion as well as the vascular volume, v.

Data analysis. The parameters $v\bar{K}$, S_t, and K_s were determined by fitting the 52 data pairs [$-F \ln (1 - E)$, P] to Eq. 1.[1] The fitting regime uses a nonlinear, iterative, derivative-free algorithm to minimize the squared residuals (5). Initial estimates of $v\bar{K}$, S_t, and K_s required by this procedure were obtained by solving Eq. 1 with three representative data pairs. The fitting regime converged in three or four iterations to the same parameter estimates for several different choices of the starting values. The calculations were carried out in double-precision arithmetic on the Amdahl mainframe at the University of Missouri Computer Center. Goodness of fit is reported as the coefficient of determination.

RESULTS

The data from 52 experiments in 26 rats appear in Fig. 1 as a plot of $-F \ln (1 - E)$ against P spanning a range

[1] The free fraction, α, is a function (derived in the Appendix) of K_s, S_t, and P as well as the independently measured parameters that govern the binding of rose bengal to albumin.

of free fractions from $\alpha = 0.001$ to $\alpha = 0.539$. The fitted curve depicts the best representation of this data by Eq. 1. The coefficient of determination, i.e., the fraction of the total variation in $-F \ln (1 - E)$ attributable to the model, is 0.89. The parameter estimates appear in Table I together with their standard deviations and the comparable estimates taken from Weisiger's study of albumin binding to isolated rat hepatocytes (3). N in this table denotes the number of albumin sites per liver cell computed from

$$N = AS_t v(1 + \gamma)/R, \quad (2)$$

where A is Avogadro's number, $v(1 + \gamma)$ is the extracellular volume per milliliter of liver volume (0.155) (6), and R is the number of liver cells per milliliter of liver volume (169×10^6) (7).

Representative binding data for the association between rose bengal and albumin appear in Fig. 2. Average values and their ranges from three such studies are for the equilibrium constant, K_L, 6.5×10^5 ($5.8 \times 10^5 - 7.8 \times 10^5$) M^{-1} and for the number of binding sites, n, 9.5 (6.9–12.3). The correlation coefficient for the linear regression is 0.99 (0.98–0.99). The binding data span a range of free fractions, $\alpha = 0.17$–0.67.

Bile production, O_2 consumption, perfusate pressure, the concentration of potassium, and the pH of perfusate, as well as hepatic morphology, as judged by light microscopy, have been reported previously for this preparation (2, 8). All of these indices of functional integrity were stable and within the normal range throughout the 40 min required to complete the present experiments.

Interpretation. The assumption that bound and free rose bengal are effectively at equilibrium with each other despite continuous removal of the latter ensures that α in Eq. 1 is assigned the largest value consistent with the binding data in Fig. 2. Accordingly, the model assigns the largest possible flux rate to the uptake of free ligand directly from extracellular fluid. Earlier reports in this series have already shown that under these circumstances the removal rates of taurocholate (1) and rose bengal (2) are two to six times faster than can be accounted for by simple spontaneous dissociation. This is the basis for the suggestion that some additional mechanism must operate to make ligand available for transport into the cell. Others have reached similar conclusions for oleic acid (3), bromsulfophthalein (9), and iopanoic acid (10). The transport data that support this conclusion, though compelling, do not in themselves address the questions of how or where the unexpected removal of bound ligand occurs. One suggestion (1) is that binding of an albumin-ligand complex to the cell surface leads to a transient conformational change in the albumin molecule releasing its ligand at a location that ensures its ultimate transport. The observation by Weisiger et al. (3) that ligand-free albumin binds to the surface of isolated hepatocytes is consistent with this idea, but does not address the question of whether binding of albumin to the cell surface is related to ligand transport. The thrust of the present experiments is to illuminate the missing link in this argument by predicting the binding kinetics at the cell surface from measurements of ligand clearance. Because the model predicts values for the number of cell surface sites and their affinity for albumin that are strikingly similar to Weisiger's, we conclude that our experiments and his describe the same phenomenon, and that the transport model we have assumed is physiologically plausible.

An important corollary implicit in this conclusion is the inference that the sites on the cell surface display a similar affinity for albumin whether or not it carries a ligand destined for hepatic uptake. This feature of the model accounts for two observations that are otherwise hard to explain. First, ligands with diverse structures, different binding affinities, and distinct uptake mechanisms (e.g., fatty acids, bile acids, rose bengal) all participate in an apparently similar mechanism. This in turn suggests that the reaction at the cell surface is not ligand specific—an economical evolutionary development that could provide for the disposal of many different albumin-ligand complexes by a single kind of cell surface site. Second, the inference that the affinity of albumin for the cell surface is independent of the ligand concentration accounts for the otherwise paradoxical finding that ligand clearance is strongly inhibited by increasing concentrations of albumin. According to the present model this effect is attributable to competition between ligand-free albumin and ligand-albumin complexes for a limited number of sites on the plasma membrane. The basis for this conclusion can be appreciated from Fig. 1. For example, a change in the bound fraction of rose bengal from 0.988 to 0.999 is associated with a reduction in the extraction fraction from 0.35 to 0.20. Because the concentration of rose bengal in afferent perfusate is the same in each case, and because in each case it is virtually totally bound to albumin, the change in the extraction fraction cannot be attributed to a change in the equilibrium distribution of free dye in extracellular fluid. It is readily accounted for, however, by competition between ligand-free albumin and albumin-ligand complexes for the same sites on the cell surface.[2]

[2] To explain our data it would presumably be sufficient to stipulate that only albumin-ligand complexes occupy the cell surface sites, because the effect of increasing the albumin

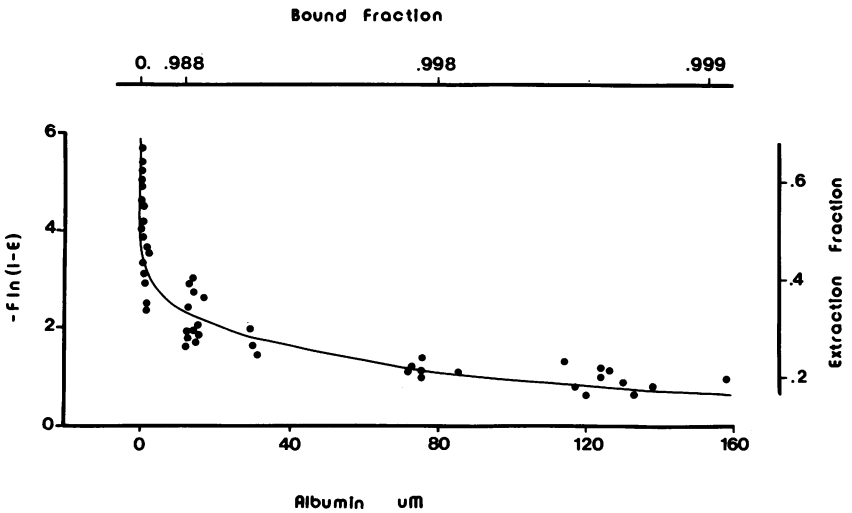

FIGURE 1 The fit of 52 data pairs to Eq. 1. Coefficient of determination using $-F \ln (1 - E)$ as dependent variable = 0.89.

The sharp reduction in the rose bengal extraction fraction associated with increasing concentrations of albumin may seem at first glance to imply that a live rat with a plasma albumin concentration substantially higher than we have used would have only a trivial capacity to remove this dye. It should be recalled, however, that the data in Fig. 1 were obtained with a perfusate flow much greater than normal hepatic plasma flow. When the fitted parameters in Table I are used to calculate the extraction fraction that would have been observed with a hepatic plasma flow of 0.7 ml min^{-1} g^{-1} and a plasma albumin concentration of 570 μM (4 g/dl), the value of E predicted by Eq. 1 is 0.33, similar to that reported for intact rats (11). We don't know whether rat albumin displays the same binding parameters for rose bengal that we have determined for bovine albumin or whether the two kinds of albumin display similar affinities for the cell surface. The calculation, nevertheless, serves to emphasize a qualitative conclusion we have suggested previously (2), namely, that the phenomenon is not species specific with respect to albumin.

DISCUSSION

The interpretation discussed above proceeds from the finding that a simple mathematical model yields parameter estimates similar to those obtained independently by an entirely different experimental approach, as well as from the good agreement between the form of the present data and the model equation. The first of these criteria is robust, but the latter is a weak basis for physiologic conclusions. It is important therefore to consider several simplifications in the model, which though mathematically convenient, may be questioned on physiologic grounds.

The model ignores the fact that perfused livers secrete newly synthesized albumin and perhaps other proteins that may bind rose bengal. It has proved impractical to identify these proteins and, in any event, their source and distribution within the liver lobule is unknown. To examine the possible effect of these uncertainties on the fitted parameters, we have measured the total protein concentration in both afferent and efferent perfusate in each rat. The fitting procedure was carried out twice, once by setting P in Eq. 1 equal to the afferent albumin concentration (Fig. 1) and a second

TABLE I
Association between Albumin and Cell Surface

	Albumin-ligand complexes (perfused liver)	Ligand-free albumin (isolated hepatocytes)
N	$10.4 \pm 1.9 \times 10^6$	$10 \pm 3 \times 10^6$*
K_s (μM)	53±13	25±7*
$v\bar{K}$ ($ml\ min^{-1}\ g^{-1}$)	7.9±0.5	—

N, sites per cell; K_s, equilibrium constant; $v\bar{K}$, intrinsic ligand clearance, mean values±SD.
* Taken from reference 3.

concentration is to reduce the average number of ligand molecules per complex. In view of Weisiger's observations, however, our model stipulates that ligand-free albumin also binds to the cell surface.

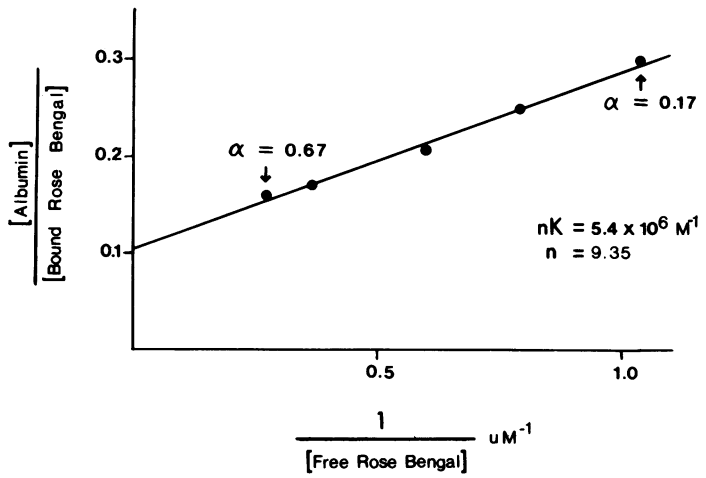

FIGURE 2 Representative binding data for the association of rose bengal with bovine albumin. K (= K_L in the Appendix), equilibrium constant. n, number of binding sites per protein molecule. α, free fraction of rose bengal.

time by assuming that all of the efferent protein was albumin, using this determination as an alternative value for P. The efferent protein concentration is on the average 24% higher than the afferent level when the latter is 1.5 μM. This difference diminishes rapidly, however, as the afferent albumin concentration increases, becoming imperceptible as the latter approaches 10 μM. The fit of the revised data is not visually different from that in Fig. 1 and yields the same coefficient of determination. The revised estimates of $v\bar{K}$, N, and S_t differ from those in Table I by 14, 7, and 10%, respectively. We suggest, therefore, that resolving the uncertainty imposed by the secretion of small amounts of endogenous protein would be unlikely to alter our conclusions.

A second simplification implicit in Eq. 1 is the assumption that the uptake of ligand liberated from albumin at the cell surface is governed by the same rate constant that determines the uptake of free ligand directly from extracellular fluid. There is no a priori reason why this should be so. On the contrary, it is easy to imagine that ligand liberated from special sites on the cell surface might enjoy a transport advantage over that engaging the transport mechanism directly from the Disse space. Unfortunately this possibility cannot be evaluated confidently with the data at hand. An equation analogous to Eq. 1 can easily be constructed to include two uptake rate constants instead of one, and in principle one can fit the data to the resulting four-parameter system. In practice, however, the solution to this more difficult estimation problem is characterized by slow convergence of the fitting regime and large standard deviations of the parameter estimates. The actual values obtained appear in Table II, where $v\bar{K}_f$ and $v\bar{K}_b$ incorporate the uptake constant for free ligand in extracellular fluid and that for ligand bound on the cell surface, respectively. The values in Table II though similar to those in Table I are determined with much less confidence. Accordingly, there is no basis for concluding that the apparent difference between $v\bar{K}_f$ and $v\bar{K}_b$ is physiologically meaningful, or that the revised estimates of N and K_s are closer to the true values than those obtained from the three-parameter fit (Table I).

TABLE II
Alternative Estimates Based on a Four-Parameter Fit

N	5.4±7.0 × 10^6
K_s (μM)	52±66
$v\bar{K}_f$ ($ml\ min^{-1}\ g^{-1}$)	7.9±0.6
$v\bar{K}_b$ ($ml\ min^{-1}\ g^{-1}$)	15±6

Kinetic parameters in Table I revised on the assumption that $v\bar{K}_f \neq v\bar{K}_b$, the intrinsic clearances of free and bound ligand, respectively. Data represent mean values±SD.

Another approach to this problem that may appear theoretically attractive but fails in practice is to determine the transport parameter in Eq. 1 directly from experiments conducted with protein-free perfusate. The model equation in this case reduces to

$$-F \ln (1 - E) = v\bar{K}_f.$$

The remaining parameters, $v\bar{K}_b$, N, and K_s, might then

be determined unambiguously from the data in Fig. 1. As pointed out above, however, protein-free perfusate is not a technically feasible objective.

In view of these difficulties the relative magnitudes of $v\bar{K}_f$ and $v\bar{K}_b$ remain uncertain pending more definitive experiments. Meanwhile, it is important to emphasize that the good agreement between our estimates of N and K_s and Weisiger's and between our data and Eq. 1 are only consistent with, rather than compelling evidence for, the assumption that $v\bar{K}_f$ and $v\bar{K}_b$ are equal.

As a third consideration it may be noted that Eq. 1 contains only one perfusate flow, in effect representing the whole liver as a single equivalent sinusoid. In fact, however, there are many sinusoids with an unknown distribution of flows. To examine this problem we have incorporated several arbitrary flow distributions into Eq. 1 and fitted the data to the resulting integral equations. The mathematical details of this procedure appear in the Appendix. We have examined the effect of two Gaussian flow distributions with coefficients of variation equal to 0.15 and 0.35, as well as the effect of two gamma distributions with third central moments (measures of skewness) equal to 1.45 and 2.58. The revised estimates of $v\bar{K}$, N, and K_s resulting from these calculations differ from those in Table I by at most 8, 20, and 17%, respectively. Because the flow distributions we have selected represent wider dispersions than do typical hepatic indicator dilution curves (8, 12), we are confident that no important variations in $v\bar{K}$, N, or K_s have been missed by modeling the liver as a single equivalent sinusoid.

Finally, it is important to consider the kinetic implications of and the justification for assuming that the binding of rose bengal to albumin and the binding of albumin to the cell surface are effectively at equilibrium everywhere along the sinusoid. The kinetic implication in each case is to ensure that the removal of ligand is limited jointly by the rate of perfusate flow and the transport rate constants, not by the velocity of the binding reactions. The equilibrium assumption seems physiologically plausible in view of the survival value of efficient ligand removal and it has the advantage of greatly simplifying the mathematics, but it cannot be certified in quantitative terms, because the individual association and dissociation rate constants are unknown. The following indirect evidence may be noted, however. The binding of small organic anions to albumin is reported to be diffusion limited with association rate constants in the range 10^7–10^{10} M^{-1} s^{-1} (13, 14). If similar values hold for rose bengal it is easy to show from the measured equilibrium constant that the time for spontaneous dissociation of the bound complex cannot be a material determinant of the observed removal rate over the range of protein/ligand ratios covered by the data in Fig. 1. The reason for this conclusion is that the time for the bound and free forms of rose bengal to achieve 99.5% of their equilibrium distribution under these circumstances is at least 10 times faster than the convective transit time or the time characteristic for net transport from the Disse space (2). It is worth noting, moreover, that for any given concentration of rose bengal, the equilibrium assumption becomes more conservative as the albumin concentration increases. For albumin concentrations > 10 μM, for example, the assumption of a diffusion limited "on" rate predicts that the time to reach 99.5% of the equilibrium distribution is shorter than the transport time by a factor of at least 100. The reason for this is that as the equilibrium concentration of free ligand diminishes, less time is required for this amount to dissociate. The importance of this to interpreting Fig. 1 is that the estimates of K_s and S_t are dominated by the data obtained at the higher albumin concentrations.

Revising the model to include the possibility of slow (i.e., nonequilibrium) binding reactions poses a difficult problem in nonlinear differential equations for which an explicit analytical solution in closed form does not exist. A numerical solution to this problem will be published separately. It may be noted here, however, that the transport data in Fig. 1 do not in themselves contain sufficient information to distinguish between the model we have assumed and an alternative formulation in which the rate-limiting transport step is the spontaneous dissociation of albumin-ligand complexes. The present argument thus depends not on the shape of the kinetic curve in Fig. 1 but on the similarity of the fitted parameters derived from it to those obtained directly by a completely different experimental approach that does not depend on ad hoc model assumptions.

Summary. Rose bengal has a hepatic extraction fraction that is unexpectedly high in view of its extensive binding to albumin. Increasing concentrations of albumin inhibit this process under circumstances where the reduced extraction cannot be attributed to changes in the equilibrium binding of this dye in extracellular fluid. These findings can be accounted for by a distributed transport model that features binding of the albumin-ligand complex to the cell surface followed by dissociation of the complex and presentation of the liberated ligand to the uptake carrier. The number of cell surface sites and their affinity for albumin predicted by this model are similar to those determined directly from the binding of ligand-free albumin to isolated hepatocytes. We suggest on this basis that the binding of albumin to the cell surface is functionally equivalent to the process that makes bound ligand available for hepatic uptake.

APPENDIX

Model for one sinusoid. Consider a single sinusoid of volume, v, perfused at flow rate, F. We define the location variable, x, as cumulative vascular volume such that x runs from zero at the portal inlet to v at the hepatic venous outlet. The binding of ligand to albumin is defined by the equilibrium

$$\hat{P} + L \overset{K_L}{\leftrightarrow} L_p \tag{1a}$$

and the conservation equations

$$\hat{P} + L_p = nP_t \tag{2a}$$

$$L + L_p = L_t, \tag{3a}$$

in which $\hat{P}$ denotes ligand-free binding sites, L denotes free ligand, L_p is ligand bound to albumin, P_t is total albumin, and L_t is total ligand. K_L (M^{-1}) is the equilibrium binding constant and n is the number of binding sites. In writing Eqs. 1a through 3a we ignore distinctions between albumin in extracellular fluid and albumin on the cell surface because both forms are assumed to bind the ligand with the same affinity. The analogous relations that govern the association of albumin with the cell surface are

$$P + S \overset{K_s}{\leftrightarrow} S_p \tag{4a}$$

$$P + S_p = P_t \tag{5a}$$

$$S + S_p = S_t. \tag{6a}$$

Here P is the albumin concentration in extracellular fluid, S_p denotes albumin bound to the cell surface, S denotes unoccupied cell surface sites, and S_t denotes total sites. Again we ignore distinctions between albumin that carries the ligand and that which is ligand-free, because sites on the hepatocyte are assumed to bind both forms with the same affinity.

The uptake of ligand from a small volume of sinusoid, Δx, consists of two terms: the uptake of free ligand directly from the Disse space and the uptake of free ligand from the cell surface. In each case we define the uptake flux as the mass of extravascular solute times an uptake rate constant, k_1. If α is the free fraction of ligand, the total uptake flux is

$$\alpha k_1 \gamma L_t(x)\Delta x + (1 - \alpha)(S_p/P_t)k_1 \gamma L_t(x)\Delta x, \tag{7a}$$

in which γ is the ratio of the Disse volume to the sinusoidal volume. The product $(1 - \alpha)(S_p/P_t)$ is the fraction of ligand bound to albumin times the fraction of albumin bound to the cell surface and is thus the fraction of ligand bound on the surface. To cast 7a in terms of measurable quantities, as well as the unknown parameters of interest, K_s and S_t, we proceed as follows.

Let u be the concentration of total ligand in extracellular fluid. The mass of ligand on the cell surface per unit of extracellular volume is then

$$L_t - u = L_p(S_p/P_t) = (L_t - L)(S_p/P_t). \tag{8a}$$

Using the definitions $\alpha = L/L_t$ and $\lambda = S_p/P_t$ equation 8a may be recast as

$$L_t = u/[1 - (1 - \alpha)\lambda]. \tag{9a}$$

Eqs. 1a, 2a, and 3a lead to

$$K_L = \frac{L_p}{\hat{P}L} = \frac{L_t - L}{[nP_t - (L_t - L)]L} = \frac{1 - \alpha}{[(nP_t/L_t) - (1 - \alpha)]L_t\alpha}, \tag{10a}$$

which for the special case where the ratio, nP_t/L_t, is much larger than 1, reduces to

$$\alpha \simeq 1/(nP_tK_L + 1). \tag{11a}$$

This approximation frees α from dependence on ligand concentration and greatly simplifies the calculations. In our experiments the lowest molar ratio of albumin to rose bengal in perfusate is 100 and n is ~9, so that the largest error encountered in using Eq. 11a is of the order of 1/900.

From Eqs. 4a, 5a, and 6a we have

$$\lambda = S_p/P_t = S_t/(S_t + K_s + P) \tag{12a}$$

$$P_t = P(K_s + P + S_t)/(K_s + P), \tag{13a}$$

which together with 9a and 11a suffice to express 7a in the desired terms. Note that K_s has molar units and is thus defined in a sense that is the inverse of that for K_L.

The differential equation for net solute removal can now be developed by incorporating the uptake flux and the analogous terms for efflux and excretion into the conservation equations. In the steady state the net flow of ligand into and out of the system must be zero. Accordingly,

$$Fu(x) + k_2 z(x)\theta\Delta x = \gamma k_1 u(x)[\alpha + (1 - \alpha)\lambda]/[1 - (1 - \alpha)\lambda]\Delta x + Fu(x + \Delta x) \tag{14a}$$

$$\alpha = (P + K_s)/[nPK_L(P + K_s + S_t) + P + K_s] \tag{15a}$$

$$\lambda = S_t/(P + S_t + K_s) \tag{16a}$$

$$\theta z(x)(k_2 + k_3) = u(x)\gamma k_1[\alpha + (1 - \alpha)\lambda]/[1 - (1 - \alpha)\lambda], \tag{17a}$$

where z(x) is the concentration of ligand inside liver cells, θ is the ratio of cell volume to sinusoidal volume and the rate constants, k_2 and k_3, govern efflux and removal, respectively.

The desired differential equation comes from dividing equation 14a by Δx, taking the limit as $\Delta x \rightarrow 0$, and using 17a to eliminate $\theta z(x)$. This procedure yields

$$-F\frac{du}{dx} = Ku[\alpha + (1 - \alpha)\lambda]/[1 - (1 - \alpha)\lambda] \tag{18a}$$

$$K = \gamma k_1 k_3/(k_2 + k_3), \tag{19a}$$

which is to be solved using the definitions 15a and 16a. The solution is

$$-F \ln (1 - E) = v\bar{K}[\alpha + (1 - \alpha)\lambda]/[1 - (1 - \alpha)\lambda] \tag{20a}$$

$$E = 1 - u(v)/u(0) \tag{21a}$$

$$\bar{K} = \int_0^v \frac{K dx}{v} = \text{average value of } \gamma k_1 k_3/(k_2 + k_3). \tag{22a}$$

The definition of $\bar{K}$ as an integral reflects our treatment of the space ratio, γ, and the transport rate constants as unspecified functions of x and thus frees the model from stipulations that the sinusoidal dimensions or the transport capacities for uptake, efflux, or excretion be uniform along the length of the sinusoid.

Model for many sinusoids. Eq. 18a can be written for a sinusoid with flow f as

$$-f\frac{du_f}{dx} = Ku_f[\alpha + (1-\alpha)\lambda]/[1-(1-\alpha)\lambda], \qquad (23a)$$

in which $u_f(x)$ is the concentration of ligand in the sinusoidal lumen. If $\Psi(f)$ denotes the probability density function of flows, the extraction fraction for a whole liver is given by

$$E = 1 - \int_0^\infty [fu_f(v)\Psi(f)/Fu(0)]df, \qquad (24a)$$

in which F is perfusate flow rate and u(0) is the inlet concentration common to all sinusoids. Eqs. 23a and 24a can be combined to yield

$$-F\ln(1-E) = -F\ln\left[\int_0^\infty \frac{f\Psi(f)}{F}\exp - \{vK[\alpha + (1-\alpha)\lambda]/f[1-(1-\alpha)\lambda]\}df\right]. \qquad (25a)$$

The data in Fig. 1 were fitted to this equation for specified choices of $\Psi(f)$ using numerical integration to evaluate the integral (Simpson's composite rule, 10 breakpoints over the interval [0, 2F]).

ACKNOWLEDGMENTS

This work was completed with the expert technical assistance of Neal Shurmantine, Frances Thompson, Kathi Keppler, and Carol Ritter.

This work was supported by National Institutes of Health grant AM27623.

REFERENCES

1. Forker, E. L., and B. A. Luxon. 1981. Albumin helps mediate removal of taurocholate by rat liver. *J. Clin. Invest.* 67:1517–1522.
2. Forker, E. L., B. A. Luxon, M. Snell, and W. O. Shurmantine. 1982. Effect of albumin binding on the hepatic transport of rose bengal: surface-mediated dissociation of limited capacity. *J. Pharmacol. Exp. Ther.* 223: 342–347.
3. Weisiger, R., J. Gollan, and R. Ockner. 1981. Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. *Science (Wash. DC).* 211:1048–1051.
4. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
5. Ralston, M. L., and R. I. Jennrich. 1978. Dud, a derivative-free algorithm for nonlinear least squares. *Technometrics.* 20:7–14.
6. Blouin, A., R. O. Bolender, and E. R. Weibel. 1977. Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. *J. Cell Biol.* 72:411–455.
7. Weibel, E. R., W. Staubli, H. R. Gnagi, and F. A. Hess. 1969. Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model, stereologic methods, and normal morphometric data for rat liver. *J. Cell Biol.* 42:68–91.
8. Luxon, B. A., P. D. King, and E. L. Forker. 1982. How to measure first-order hepatic transfer coefficients by distributed modeling of a recirculating rat liver perfusion system. *Am. J. Physiol.* 243:518–531.
9. Weisiger, R., C. Zacks, N. Smith, and J. Boyer. 1982. Hepatic uptake of ^{35}S-sulfobromophthalein (BSP) from albumin solutions in elasmobranchs. *Gastroenterology.* 82:1249 (Abstr.)
10. Barnhart, J. L., B. Witt, W. Hardison, and R. Berk. 1983. Uptake of iopanoic acid by isolated rat hepatocytes in primary culture. *Am. J. Physiol.* 244:630–636.
11. Meurman, L. 1960. On the distribution and kinetics of injected I-131 rose bengal. *Acta. Med. Scand.* 167:7–85.
12. Goresky, C., G. G. Bach, and B. E. Nadeau. 1973. On the uptake of materials by the intact liver. The transport and net removal of galactose. *J. Clin. Invest.* 52: 991–998.
13. Jardetsky, O. 1966. The study of drug-binding sites by nuclear magnetic relaxation. *Proc. Int. Pharmacol. Meet.* 7:189–191.
14. Eigen, M., and G. G. Hammes. 1963. Elementary steps in enzyme reactions. *Adv. Enzymol.* 25:1–38.